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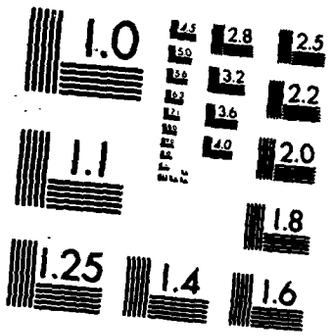

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The purpose of this project was to understand the mechanisms by which use-dependent changes in synaptic transmission can encode information into neural networks. Our working hypothesis that guided this effort was that long-term synaptic potentiaionn (LTP) is an excellent candidate mechanism for information storage in the nervous system. The project was organized around three interrelated efforts. First, new experimental and theoretical techniques for analyzing synaptic function were developed. Second, these and conventional methods were used to understand the biophysical and molecular mechanisms responsible for LTP in several different tissues. Third, the relationship between LTP and several formal information encoding schemes was demonstrated. These included synaptic analogs to classical conditioning, Hebb's postulate, and a modified version of Klopf's postulate. The results add confidence to our working hypothesis; they provide new insights into our understanding of synaptic plasticity; and they will enable the definitive tests of some leading theories.

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I. OBJECTIVES OF THE RESEARCH EFFORT

One of the prize goals of neurobiology is to understand the neurophysiological and biophysical mechanisms underlying memories and habits. A well-motivated hypothesis is that learning involves some change in the functional connectivity among nerve cells, probably at their synaptic interconnections. A major purpose of the present project was to quantify the laws governing long-term, use-dependent, synaptic plasticity and to understand the underlying cellular and biophysical mechanisms. A related goal was to infer how the empirical laws governing the synaptic modifications might explain forms of associative learning in higher organisms. Variations of Hebb's and Klopf's postulates for learning were tested, to determine whether the required physiology is present at the synaptic level, and an effort was made to determine whether synapses can undergo modifications that could be considered simple analogs of classical conditioning.

II. STATUS OF RESEARCH EFFORT

Long-term synaptic potentiation (LTP) is a use-dependent form of enhanced synaptic efficacy that can persist for hours or longer and can be induced by activation of the synapses for only a few seconds or less. This great asymmetry between the duration of the synaptic activity and the duration of the subsequent synaptic change is the defining characteristic of LTP--a property that makes this phenomenon an interesting possible mechanism for long-term control of information flow through adaptive neural networks.

For experimental purposes, it is useful to distinguish between the expression and the induction of LTP (Briggs, Brown, and McAfee, 1985; Baxter, Bittner, and Brown, 1985; Barrionuevo, Kelso, Johnston, and Brown, 1986). What follows summarizes progress made in understanding the underlying neurophysiological and biophysical mechanisms responsible for the expression and induction of LTP. New information about the conditions, rules or laws that describe the occurrance of LTP is also summerized. The latter type of information is valuable because it provides insights into the possible role of this synaptic modification as a substrate for learning in adaptive neural networks.

A. Expression of LTP. The project addressed the fundamental question: What is the proximal neurophysiological or biophysical cause of the enhanced synaptic efficacy observed during LTP? The first step in the analysis distinguished among five categories of possible mechanisms (Fig. 1, TIER 1). All five were tested (Barrionuevo and Brown, 1984; Griffith, Brown, and Johnston, 1984; Barrionuevo, Kelso, Johnston, and Brown, 1986; Griffith, Brown, and Johnston, in press). The following mechanisms were rejected or failed to receive confirming support from these experiments: (1) An increase in the postsynaptic input resistance or the effective input impedance seen by a synaptic current waveform; (2) An increase in the postsynaptic excitability (a decrease in the postsynaptic spike threshold); (3) A decrease in the peak conductance produced by the synaptic inhibition that normally accompanies synaptic excitation; (4) A modification of the ionic selectivity property of those postsynaptic channels responsible for the excitatory synaptic response, resulting in a positive shift in the equilibrium potential. The remaining possibility, which was confirmed, is that hippocampal LTP results from an increase in the measured conductance produced by the monosynaptic excitatory input.

pendent tests of the new analytical method. This new preparation is based on the cultured slice method of Gahweiler. To this will be added computer-enhanced video microscopy, which should permit clear visualization of the neurons. Better visualization will in turn enable experimental manipulations that were previously impossible. The reason for placing so much emphasis on performing a meaningful quantal analysis of LTP in the hippocampus is that the viability of the major hypotheses for LTP all hinge on the outcome of these experiments. It is therefore crucial that the results be unequivocal. More generally, the ability to perform convincing quantal studies on vertebrate CNS synapses has widespread application in neuropharmacology, neurophysiology, and biophysics.

B. Induction of LTP. Regardless of how LTP is ultimately expressed, we still need to know the conditions that bring it about and the causal sequence of events that initiate the modification. Even if the expression of LTP proves to be due to a presynaptic modification, as is known to be true in the crayfish neuromuscular junction, this does not rule out the possibility that the postsynaptic side of the cleft participates in a crucial aspect of the induction step (Baxter, Bittner, and Brown, 1985). Should the latter possibility prove to be the case--and this is currently our working hypothesis--then there may be a fascinating and previously undiscovered form of retrograde synaptic control.

Understanding the induction step is crucial for appreciating the conditions under which LTP might be expected to occur. Knowledge about the conditions that cause LTP induction is essential for inferring its possible role in associative learning. In certain regions of the hippocampus, LTP is known to have an associative property (Barrionuevo and Brown, 1983). A provocative question arose: How similar are the features of associative LTP and the known laws of classical (Pavlovian) conditioning? As a first step in addressing this question, Kelso and Brown (1986) applied to hippocampal synapses stimulation paradigms that share formal similarities to Pavlovian conditioning. Bower (1986) recently summarized these experiments in an article written for Science News, entitled "Conditioning stirs 'synaptic memory'". This article--written for the nonspecialist--describes the background and motivation to the problem addressed by Kelso and Brown in a clear and concise manner:

> The hippocampus, a small bundle of cells deep in the brain, plays an important role in making the learned associations that characterize classical conditioning. When rabbits, for example, are simultaneously presented with a tone and an air puff aimed at the eye, the activity of pyramidal cells in this brain region increases before the animals learn to blink their eyes in response to the tone alone; pyramidal cell activity does not increase when the air puff and tone are presented separately (SN; 12/10/83, p. 380).
>
> A similar type of conditioning has now been observed in rats, in the synapses that transmit nerve impulses to the same hippocampal cells. Since there is a form "synaptic memory" in the hippocampus, say Stephen R. Kelso and Thomas H. Brown of the Beckman Research Institute of the City of Hope in Duarte, Calif., it may mediate simple types of learned associations. Learned associations involving more

than one conditioning stimulus can be used with the synapses to see if cellular changes run parallel to similarily produced behaviorial responses, they report in the April 4 SCIENCE.

In the article in Science magazine, to which Bower (1986) was referring, Kelso and Brown (1986) summarized their conclusions on differential conditioning of associative LTP as follows:

> We have shown that (i) this is an activity-dependent form of neuroplasticity; (ii) the induction of the functional modulation is rapid; (iii) the expression of the enhanced synaptic strength is persistent; (iv) modification of one synaptic input is conditionally dependent on temporal contiguity or contingency with activity in another synaptic input to the same region; and (v) the associative enhancement is specific to synapses whose activity conforms to the temporal requirement. These are also features of the synaptic interactions in identified circuits of Aplysia that have been demonstrated to mediate behaviorial differential Pavlovian conditioning.
>
> A reasonable working hypothesis is that the mechanism responsible for these plastic properties of hippocampal synapses participates in some aspect of the suspected role of this cortical circuitry in higher-order Pavlovian conditioning. The occurrance of this form of synaptic memory in the hippocampal brain slice will enable investigation of associative interactions at the level of synaptic microphysiology and biophysics. Finally, differential conditioning paradigms can be used to determine the extent to which synaptic modification roles parallel those of higher-order conditioning. (quoted with numbered references and notes deleted)

These experiments demonstrated that associative LTP in the hippocampal formation has precisely those features that one might expect of a synaptic modification that participates in some aspect of associative conditioning. The results add support to the working hypothesis that some such form of use-dependent synaptic plasticity serves as the basis for encoding information into adaptive neural networks within the mammalian central nervous system (CNS).

Further support for this working hypothesis was provided by the demonstration of a Hebb-like mechanism underlying associative LTP in these synapses. For years, theoreticians interested in learning in adaptive neural networks have postulated the existance of Hebbian synapses. Such theoretical studies have shown that neural networks interconnected by Hebbian or Hebb-like synapses are indeed capable of rather interesting forms of adaptive modifications. However, for experimental neurophysiologists, a nagging question has persisted: Do Hebbian synapses actually exist? Kelso, Ganong, and Brown (1986) addressed this question directly in a series of experiments that gave unequivocal results. Their experiments provided direct insight into the nature of the biophysical conjunctive mechanism that enables associative LTP in hippocampal synapses.

They demonstrated that LTP only occurs in a repetitively stimulated synapse if activity in that synaptic input to a neuron occurs at approximately the same time that the postsynaptic cell is depolarized by a critical amount (Fig. 2, TIER 1). This result--precisely what is predicted and required by Hebb's postulate for learning--was demonstrated by applying a combination of current- and voltage-clamp techniques that Dr. Brown's laboratory helped to pioneer.

The experiments found that synaptic stimulation failed to induce LTP if a voltage-clamp was applied to the postsynaptic neuron--preventing it from depolarizing during the synaptic stimulation. The experiments further demonstrated that the same synaptic stimulation did lead to the induction of LTP when a current-clamp was used to force the postsynaptic cell to depolarize by a critical amount during the synaptic stimulation. This same postsynaptic depolarization was without effect if the synaptic input was not stimulated at about the same time as the depolarization. These experiments showed that the Hebb-like conjunctive mechanism is sufficient to explain the spatiotemporal features of associative LTP reported by Kelso and Brown (1986).

As indicated above, the conjunctive mechanism revealed by these experiments is very similar to what has come to be known as Hebb's postulate for learning. However, Kelso, Ganong, and Brown (1986) stopped short of concluding that these were actually Hebbian synapses. According to Hebb's postulate for learning, the essential postsynaptic electrogenic event involves "firing" the target neuron. This is usually taken to mean that a sodium action potential must be elicited in the postsynaptic cell. Kelso, Ganong and Brown (1986) found that the elicitation of a postsynaptic sodium action potential is not necessary for the conjunctive mechanism to operate (Fig. 2, TIER 2). They suggested that the key postsynaptic component of the conjunctive mechanism may involve calcium influx. What these experiments demonstrated decisively is that a Hebb-like conjunctive mechanism exists and it can account for the known spatiotemporal properties of associative LTP.

Under certain conditions, the Hebb-like mechanism yields results that satisfy predictions of Klopf's postulate for learning. The latter was originally cast in terms of "operant" ("instrumental") conditioning, while I have tended to think more in terms of Pavlovian conditioning; yet the differences are more apparent than real. Indeed, Klopf's more recent (unpublished) mathematical formulations are in fact explicitly done from the perspective of Pavlovian conditioning. This year I spent a few days in Harry Klopf's laboratory, observing his simulations and debating the adequacy of various types of synaptic modification rules.

We agree that Pavlovian conditioning provides a useful framework both at the cellular and systems levels and that the ordinary form of Hebb's postulate has serious deficiencies, some of which were easily demonstrated through Klopf's computer simulations. We further agree that it is essential to understand how nature solves the problem and that, from the perspective of building artifical adaptive networks, it may be possible to improve on nature's solution. We do not know whether Klopf's most recent set of synaptic modification rules are actually incorporated at individual synapses or instead whether these computational capabilities only emerge at the network level. In the latter case, Klopf may have discovered how to improve on nature in a manner that should be easily and efficiently transportable into hardware implementations.

These preceeding neurophysiolgical observations provide useful insights into the possible molecular mechanisms involved in the induction step. They help formulate tentative answers to a number of questions. What is the molecular conjunction or AND-gate that controls the first step in the induction process? Is it possible that the biophysical properties of a single type of macromolecule can explain the conjunction? What biophysical properties would be required? An example of a possible answer to these questions is evident in the voltage-and agonist-dependence of the iontophore associated the N-methyl-D-aspartate (NMDA) receptor. Brown's laboratory (in preparation) has found that pharmacological agents that block the NMDA receptor also block the Hebb-like conjunctive mechanism in hippocampal synapses (Ganong and Brown, unpublished). Wigstrom and co-workers have recently published similar results. Very recent findings from Stevens' laboratory indicate how the NMDA receptor-ionophore complex could admit calcium into the postsynaptic structure in a fashion that is both voltage and transmitter dependent.

It is now possible to put forth an hypothesis that synthesizes our computer simulations of dendritic spines, our neurophysiological results, and some of the recently discovered biophysical details about the NMDA receptor-iontophore complex. Stevens (unpublished) has found that the NMDA receptor-associated channel opens to one of three conductance states, which we can call small, medium, and large. In addition to size, the large conductance state differs from the other two states in three repects. First, it is selectively opened by NMDA receptor agonists but not by kainate or quisqualate receptor agonists, which cause the channel to open to the lower conductance states. Second, the large conductance state is highly permeable to calcium ions, whereas the two lower conductance states are permeable mainly to sodium and potassium ions. Third, the probability of channel opening to the high conductance state is steeply voltage-dependent. Specifically, when there is a large, inside-negative potential across the membrane containing the channel, the high conductance state is blocked by extracellular magnesium ions. A reduction of this inside-negative potential relieves the magnesium block, thereby enabling an NMDA receptor agonist to open the channel to the large conductance state. The voltage-dependence is lost if magnesium ions are excluded from the outside of the membrane.

As we shall see below, these properties are precisely what is needed to build a model that can account for the known features of the Hebb-like conjunctive mechanism that is responsible for associative LTP. Associative LTP refers to the following: Repetitive stimulation of a weak synaptic input to a neuron fails to induce LTP in that input unless the stimulation is paired with nearly simultaneous depolarization of the cell by a separate, strong, synaptic input; yet stimulating the latter, by itself, does not induce LTP in the weak input (Barrionuevo and Brown, 1983; Kelso and Brown, 1986). The essential contribution of the strong synaptic input is simply the extra depolarization that it furnishes (Kelso, Ganong, and Brown, 1986). Thus the same associative effect is achieved when direct depolarization of the cell, through a microelectrode, is substituted for the strong synaptic input (Kelso, Ganong and Brown, 1986). In either case, the conjunctive mechanism is specific to just those synapses that were stimulated at the same time as the strong depolarization (Kelso and Brown, 1986; Kelso, Ganong, and Brown, 1986).

Our spine simulations have helped us understand the conditions under which the preceding facts can be synthesized into a single, unifying, working hypothe-

sis. One concrete version of this working hypothesis, which I am currently entertaining, postulates the following: (1) Calcium must bind to a site within the spine head in order for LTP to be induced; (2) The normal trigger for the induction step is calcium influx through the membrane of the spine head; (3) The calcium influx through the spine head is mediated via the high conductance state of the NMDA-associated channel; (4) The spine head contains no other calcium channels, or too few to trigger the calcium-dependent step; and (5) The intracellular buffering of calcium is normally so effective that calcium entering the cell through channels that are not located on the spines never reaches the essential site in the spine head. Although I have not yet done computer simulations of this specific hypothesis, I believe that it, or a closely related alternative hypothesis, may offer the most parsimonious account of the known facts.

What our computer simulations have suggested is that this hypothesis will only work if the actual peak conductance change produced by a single synapse on the head of any given spine is small (< 5 nS). If the single quantal conductance is about 1 nS, and if individual synapses normally tend to release an average of 1-2 quanta per nerve impulse, then the depolarization produced by a single synapse on the head of a spine would be insufficient to relieve the magnesium block of the high-conductance state. Under these conditions, stimulation of a small number of afferent inputs to a neuron probably would not induce LTP; especially if the synapses were located on different branches of the dendritic tree, which would cause less than linear (additive) summation of potential. Indeed, stimulation of a small number of afferents to a hippocampal neuron invariably fails to induce LTP in that input. On the other hand, if the single quantal conductance is about 5 nS, and if the synapses normally tend to release 3-5 quanta per nerve impulse, then the synaptic depolarization produced by activity in a single synapse on the spine head should be sufficient to relieve the magnesium block. Under these conditions, stimulation of a single afferent input to a hippocampal neuron would induce LTP in that input--which apparently does not occur in regions of the hippocampal formation reported to display associative LTP.

As stated earlier, although I need to do computer simulations to make a convincing argument regarding the plausibility of the working hypthesis, it is already possible to see intuitively that this hypothesis could account for many of the known neurophysiological facts about LTP induction in the hippocampal formation. Some of the key facts that can be organized around this working hypothesis are listed and (where necessary) explained below:

(1) LTP induction is normally dependent on the presence of extracellular calcium. The reason follows directly from the initial assumptions. The hypothesis makes the testable prediction that it should be possible to induce LTP in the absence of extracellular calcium--for example, by preventing intracellular calcium buffering and causing calcium release from intracellular stores. Injecting the cell with dinitrophenol (DNP) or other mitochondrial poisons might accomplish this.

(2) Repetitive stimulation of a weak synaptic input (small number of afferents) to a neuron does not result in LTP induction. The reason is that the resulting depolarizing is not sufficient to relieve the magnesium

block of the high conductance state (because this is a weak synaptic input).

(3) Stimulation of a strong synaptic input (large number of afferents) to a neuron does result in LTP induction. The reason is that the strong input causes sufficient depolarization to relieve the magnesium block of the high conductance state.

(4) A minimum "threshold" number of afferent inputs to a neuron must be stimulated for LTP induction to occur. This has been called the "cooperativity" requirement for LTP. The reason for "cooperativity" is simply the combination of the preceding two explanations.

(5) Induction of LTP in one synaptic input does not cause LTP in other, separate, inputs to the same hippocampal neuron. LTP is specific to the stimulated input; heterosynaptic LTP does not occur, at least in hippocampal region CA1 and the dentate gyrus. The proposed reason for the input specificity is that intracellular calcium buffering is sufficient to prevent calcium from diffusing to the spine heads of unstimulated synapses. The testable prediction of the hypothesis is that injection of cells with agents such an DNP that impair calcium buffering should enable heterosynaptic LTP in those cells.

(6) Postsynaptic depolorization does not induce LTP, even though such depolarization does cause calcium influx and it does enable LTP in simultaneously active synapses. The reason depolarization alone fails to induce LTP is that the resultant calcium influx fails to reach the spine heads (due to buffering). The explanation is the same as that proposed for the lack of heterosynaptic LTP. In the presence of DNP, depolarization alone might be sufficient. For that matter, extremely intense and prolonged depolarization might be sufficient even in the absence of agents like DNP. The reason depolarization enables LTP in active but weak synaptic input synapses is that it relieves the magnesium block of the high conductance state.

(7) Repetitive stimulation of a weak synaptic input to a hippocampal neuron will induce LTP in that input if the stimulation is temporally paired with strong depolarization supplied by other, separare, strong synaptic input to the same neuron. This phenomenon, called associative LTP, is specific to just those synapses that are active during the strong depolarization. The reason is that the depolarization supplied by the strong synaptic input relieves the magnesium block and enables calcium influx into the spine heads of all synapses that are simultaneously active.

(8) The induction of LTP conforms to a Hebb-like synaptic modification rule, the mechanism underlying which can account for the so-called "cooperativity requirement" for LTP as well as the known spatiotemporal properties of associative LTP. The reason for the Hebb-like synaptic modification rule is self-evident from the preceding explanations.

(9) NMDA receptor antagonists, such as APV, that block the high conductant state also block the Hebb-like conjunctive mechanism. The reason according to the proposed model is obvious.

(10) Injecting a cell with the calcium chelator EGTA reduces the probability of LTP induction. The reason is evident from the foregoing.

At the moment I am devising alternative hypotheses that are also consistent with the known facts but that lead to different new predictions. I hope to use computer simulations to explore the implications of these hypotheses for LTP induction. My laboratory is now capable of providing quantitative tests of the predicted outcomes.

C. Induction-expression coupling. At the moment, it is experimentally prudent to treat the expression and induction of LTP as separate problems, even though they are obviously connected. In order to construct well-motivated and testable hypotheses that couple induction to expression, more information is needed. We need to understand the beginning (induction) and end (expression) points before attempting to link them together. For example, if the expression of LTP involves a presynaptic modification that causes more transmittor release, and if the induction step is controllable through postsynaptic manipulations, then the coupling of induction to expression will involve some form of retrograde synaptic control. Although this is a fascinating and novel possibility, it would be unwise to make a major investment into testing this retrograde control hypothesis until there is some basis for motivating its feasability or likelihood. Experiments that are currently underway may provide such motivation. If so, it will be possible to design experiments aimed at testing specific hypothesis that couple the induction step to the expression step.

D. Strong inferences about LTP. Progress in understanding the phenomenon of LTP has been hampered by a lack of rigorous thinking and even less rigorous experimentation. The seeds of the problem and its solution are contained in an excellent article written by John Platt (Science 146,349-353, 1964), entitled "Strong Inference". Platt addresses the question, "Why should there be such rapid advances in some fields and not in others?" The flavor of his answer is contained in the following quotation:

> I think the usual explanations that we tend to think of--such as the tractability of the subject, or the quality or education of the men drawn into it, or the size of the research contracts--are important but inadequate. I have begun to believe that the primary factor in scientific advancements is an intellectual one. These rapidly moving fields are fields where particular method of doing scientific research is systematically used and taught, and a cumulative method of inductive inference that is so effective that I think it should be given the name of "Strong Inference." I believe it is important to examine this method, its use in history and rationale, and to see whether other groups and individuals might learn to adopt it properly in their own scientific and intellectual work.
>
> In its separate elements, strong inference is just the simple and old-fashioned method of inductive inference that goes back to Francis Bacon. The steps are familiar to every college student and are practiced, on and off, by every scientist. The difference comes in their systematic application. Strong inference consists of applying the following steps to every problem in science, formally and explicitly and regularly:

1) Divising alternative hypotheses;
2) Divising a crucial experiment (or several of them), with alternative possible outcomes, each of which will, as nearly as possible, exclude one or more of the hypotheses;
3) Carrying out the experiment so as to get a clean result;
1') Recycling the procedure, making subhypotheses or sequential hypotheses to refine the possibilities that remain; and so on.

It is like climbing a tree. At the first fork, we choose--or, in this case, "nature" or the experimental outcome chooses -- to go to the right branch or the left; at the next fork, to go left or right; and so on. There are similar branch points in a "conditional computer program," where the next move depends on the result of the last calculation. And there is a "conditional inductive tree" or "logic tree" of this kind written out in detail in many first-year chemistry books, in the table of steps for qualitative analysis of an unknown sample, where the student is lead through a real problem of consecutive inference . . .

The research in my laboratory has attempted to follow this method of "strong inference". The logic tree that has grown from this method is illustrated in simplified form in Figs. 1 and 2. The next round of experiments will enable my laboratory to provide the crucial information regarding the essential choice points in this logic tree. Platt provides a spirited argument to government funding agencies ". . . to put your money on ..." researchers who are devotees and practitioners of this method of strong inference. In the field of neurophysiology, I believe that this is sound advice.

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IV. FIGURES

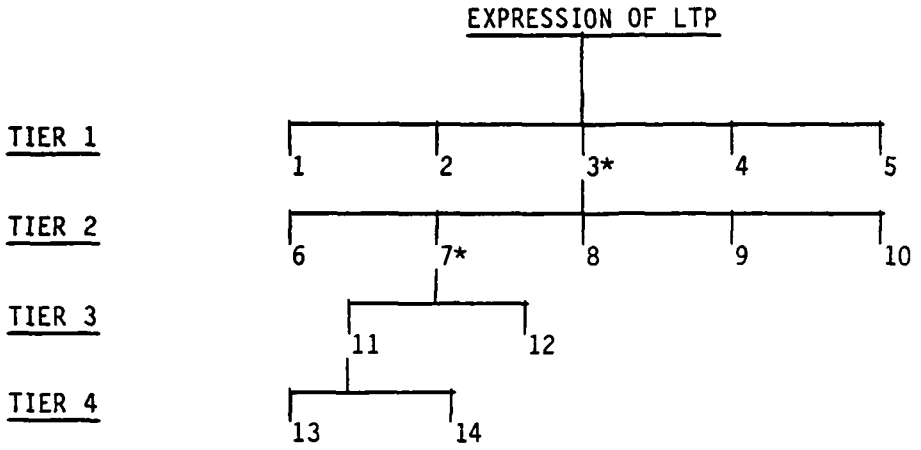

Fig. 1. Simplified partial logic tree for analysis of the neurophysiological mechanism responsible for the expression of LTP. (The actual logic tree that I use, which is somewhat more "logical" and considerably more complicated, was less easily constructed on the wordprocessor; but the illustrated one gives the flavor.) The testing of possible mechanisms in TIER 1 has been completed. Numbers in TIER 1 refer to the following possible mechanisms for the enhanced synaptic efficacy: (1) An increased postsynaptic excitability (a reduced spike threshold); (2) A positive shift in the equilibrium potential for the excitatory synaptic input; (3) An increased measured excitatory postsynaptic conductance; (4) A decrease in the conductance associated with concomittant synaptic inhibition; and (5) An increase in the postsynaptic input resistance or impedance. Mechanisms 1, 2, 4, and 5 were found to be false or received no supporting evidence. Mechanism 3 was confirmed. Present research efforts are directed at TIER 2. Numbers refer to the following possibilities: (6) A decrease in the spine axial resistance; (7) An increase in presynaptic secretion; (8) An increase in the number of postsynaptic receptors for the neurotransmitter substance; (9) An increase in the single channel conductance associated with postsynaptic receptors for the neurotransmitter substance; and (10) An alteration that increases the probability that a released transmitter molecule binds to a postsynaptic receptor for the neurotransmitter substance. Mechanisms 6, 8, 9, and 10 predict that LTP results from an increase in the mean quantal size (single quantal conductance) with no change in the mean quantal content (average number of quanta discharged per nerve impulse). Mechanism 7 makes the reverse prediction. Work currently underway is testing these predictions. The planning of hippocampal experiments for TIERS 3 & 4 will obviously depend on the outcomes of the quantal analysis. For the crayfish neuromuscular junction TIER 3 involves a binomial quantal analysis using the loose patch-clamp technique. Numbers refer to the following: (11) An increase in the number (n) of functional quantal release sites and (12) An increase in the probability (p) that an action potential discharges a quantum at any given release site. Indirect evidence favors possibility 11. TIER 4 for the crayfish neuromuscular junction is currently being constructed. Numbers refer to the following: (13) Unsilencing of previously silent release sites and (14) Physical growth of new release sites.

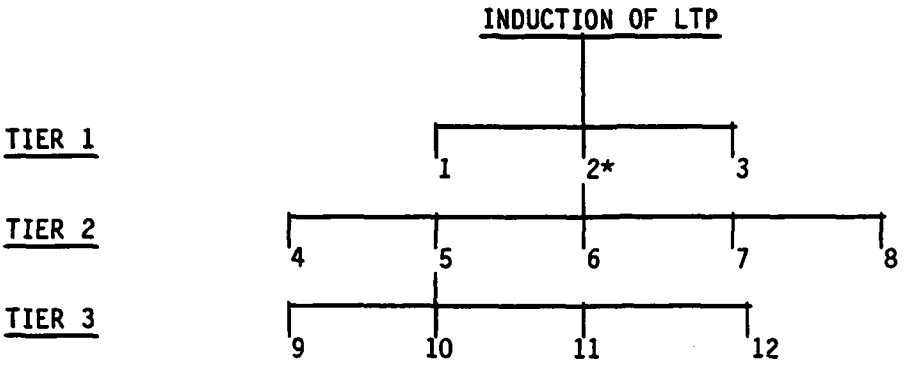

Fig. 2. Partial logic tree for analysis of the neurophysiological mechanism responsible for induction of LTP. Experimental testing of TIER 1 has been completed for the hippocampus in region CA1. Numbers refer to the following possible conditions that must be satisfied for LTP induction: (1) Presynaptic stimulation is a sufficient condition for induction; (2) Postsynaptic electrogenesis is a sufficient condition; and (3) LTP induction normally requires both presynaptic activation and postsynaptic electrogenesis. Possiblities 1 and 3 have been rejected; possiblity 2 has been confirmed. This is a Hebb-like conjunctive mechanism--one that can account for the known spatiotemporal features of associative LTP. Experiments pertinent to TIER 2 have just begun. Numbers refer to the following possible neurophysiolgical mechanisms: (4) The essential postsynaptic component of the conjunctive mechanism involves sodium spikes or sodium influx; (5) The essential event involves calcium spikes or calcium influx; (6) The event involves potassium efflux; (7) The event involves chloride efflux; (8) None of the preceding ion fluxes is essential--there is a novel second messenger. Possibility 4 has been rejected and there is circumstantial evidence implicating possibility 5. Experiments pertinent to TIER 3 are now in the planning stage. Numbers refer to the following hypotheses: (9) The essential calcium influx is normally mediated by the NMDA receptor-iontophore complex, which is located on the spine head; (10) Same as 9 but the NMDA receptor-iontophore complex is not located on the spine head; (11) Same as 9 but the NMDA receptor-iontophore complex is not the exclusive source of calcium influx and (12) Same as 11 but the NMDA receptor-iontophore complex is not on the spine head. We hope to show, through computer simulations, that possibility 9 will work and that it provides the simplest and most elegant explanation. Bliss has rejected possibility 9 in favor of 10, a decision that based on what we believe are erroneous calculations regarding the effects of dendritic spines.

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